<Original Articles>Expression of Epidermal Growth Factor Receptor after Partial Pancreatectomy in Adult Rats : An Immunohistochemical Study

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原　　著

Expression of Epidermal Growth Factor Receptor after Partial Pancreatectomy in Adult Rats: An Immunohistochemical Study

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Abstract

The expression and localization of epidermal growth factor (EGF) were investigated immunohistochemically using an anti-EGF receptor antibody in the pancreas of partial pancreatectomized and sham-operated adult rats.

In the sham-operated pancreas, immunoreactive products against EGF receptor were only slightly positive in the pancreatic acinar cells. In the partial pancreatectomized pancreas on the fifth day after operation, EGF receptor immunoreactivity was intensely positive in the acinar cells, in some cells lining the intercalated ducts and some basal cells of the acinus, but it was not detected in the pancreas when exogenous EGF was given to the rat after partial pancreatectomy for three days.

The present results suggest that EGF receptor is expressed in the regenerating pancreatic tissue, and EGF could be involved in the mechanism of pancreatic regeneration in rats.

Introduction

Epidermal growth factor (EGF) is a polypeptide consisting of 53 amino acid residues which was originally isolated from the submaxillary gland of adult male mice in 1962[7]. Studies on EGF have revealed various kinds of biological effects[5] including stimulation of cell proliferation, differentiation, and regeneration. In addition, EGF has been detected in various tissues such as the stomach, duodenum, pancreas, and kidney[14]. Ullrich and colleagues[23] determined the amino acid sequence of human EGF receptor. EGF receptor has been proven to be localized in the pancreatic acinar cells[16], but no immunohistochemical study has been reported concerning the expression of EGF receptor in the regenerating pancreas. In the present study, the expression of EGF receptor was examined immunohistochemically during the regeneration process of the pancreas after partial pancreatectomy to determine whether EGF could be involved in the regeneration mechanism of the pancreas.

Key words: Epidermal growth factor receptor, Epidermal growth factor, Pancreatectomy, Regeneration, Immunohistochemistry.
索引語：上皮細胞増殖因子，上皮細胞増殖因子受容体，膵切除術，再生，免疫組織化学
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Materials and Methods

Animals

Twenty male Sprague-Dawley rats weighing between 450 g to 500 g (aged four months) were purchased from the Japan SLC Co. (Shizuoka, Japan). The rats were housed in an adequate temperature on a 12-h light-dark cycle with free access to water and a standard pellet diet in the Institute of Laboratory Animals, Faculty of Medicine, Kyoto University.

Drugs

Recombinant human EGF was a kind gift from Earth Chemical, (Hyogo, Japan). A polyclonal anti-human EGF receptor rabbit antibody was kindly supplied by Dr. AKIYAMA. This antibody was produced against synthetic peptides corresponding to the amino acid residues 1173 to 1186 in human EGF receptor, which were part of the autophosphorylation sites in the intracellular part of EGF receptor (Fig. 1)[13], and was purified by affinity chromatography by AKIYAMA and colleagues[1,2,3]. In our previous study, the specificity of this antibody for rat EGF receptor was confirmed in the positive control and by an absorpition test[18].

Surgery

The rats were anesthetized with pentobarbital (30 mg/kg body weight) given intraperitoneally. Distal pancreatectomy was performed on twelve rats. Briefly, the procedure consisted of removal of the splenic segment of the pancreas as defined by RICHARDS and colleagues[20] using a modification of the techniques of SCOW[21] and MIGLIORINI[17]. The cut end of the pancreas was closed with silk ligation. The fascia-peritoneal layer and the skin layer were respectively closed by using interrupted suture with silk. The operation was performed to leave an approximately 50% of total organ in wet weight (Fig. 2). In the eight sham-operated rats, the pancreas was exposed and rubbed between fingertips for a few minutes.

Treatment

On the following day of surgery, the rats were randomly assigned to one of the two subgroups. Later rats were allocated into the following four groups: Group A; four rats sham-operated and injected with saline solution, Group B; four rats sham-operated and injected with 100 μg/kg EGF, Group C; six rats pancreatectomized and injected with saline solution, Group D; six rats pancreatectomized and injected with 100 μg/kg EGF. The rats in groups B and D were given intramuscularly recombinant human EGF resolved in physiological saline solution three times a day (at 8:00, 17:00, and 24:00) for three consecutive days. The rats in Group A and C were given only saline solution intramuscularly.

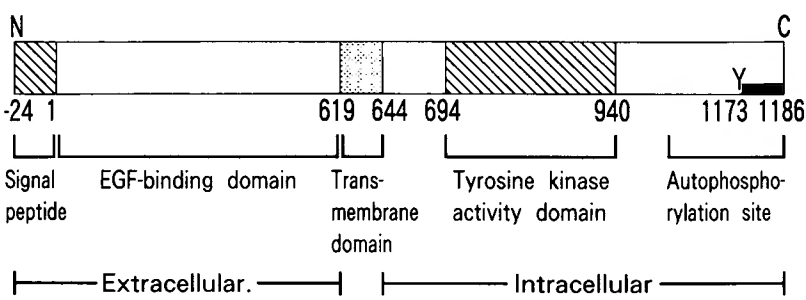
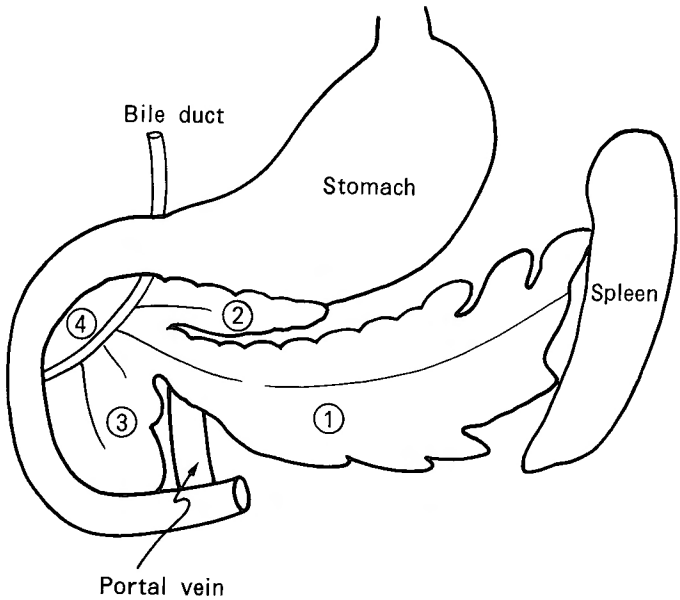


Fig. 1 Structure of EGF receptor[13].
Bar indicates the region of the synthetic peptides against the polyclonal antibody used in this study.



① : splenic segment : 50 %
② : gastric segment : 20 %
③ : duodenal segment : 20 %
④ : parabiliary segment : 10 %

Fig. 2 Scheme of the rat pancreas showing the percentage of each segment in wet weight.

Immunohistochemistry

On the fifth day after operation, all the rats were sacrificed and the pancreata were obtained for immunohistochemical studies. A modification of immunoglobulin enzyme bridge technique (ABC method) was employed[12,22]. The duodenal segments of the pancreas were fixed in 90% ethanol for 48 hrs and embedded in paraffin. Deparaffinized sections (5 μm thick) were immersed in 70% methanol containing 0.3% hydrogen peroxide for 30 min to block the endogenous peroxidase activities, and incubated with non-immunized normal goat serum (diluted to 3%) for 30 min in order to block the nonspecific Fc receptor binding reactions. The sections were treated consecutively at room temperature with polyclonal anti-human EGF receptor rabbit antibody (diluted 1 : 1,000 with PBS containing 1% BSA) for overnight, biotinylated anti-rabbit IgG goat antiserum (diluted 1 : 200; Vectastain, *Elite* ABC Kit, Vector Laboratories, Inc., California, USA) for 30 min, and avidin dehydrogenase-biotinylated horseraddish peroxidase complex (Vectastain *Elite* ABC Kit) for 30 min. Peroxidase staining was performed for 5 min using a solution of 10 mg of 3,3′-diaminobenzidine tetrahydrochloride in 100 ml of 50 mM Tris-HCl (pH 7.6) containing 0.02% hydrogen peroxide[10]. The cell nuclei were counterstained with 1% methylgreen. The control sections were treated with normal rabbit serum instead of anti-human EGF receptor antibody.

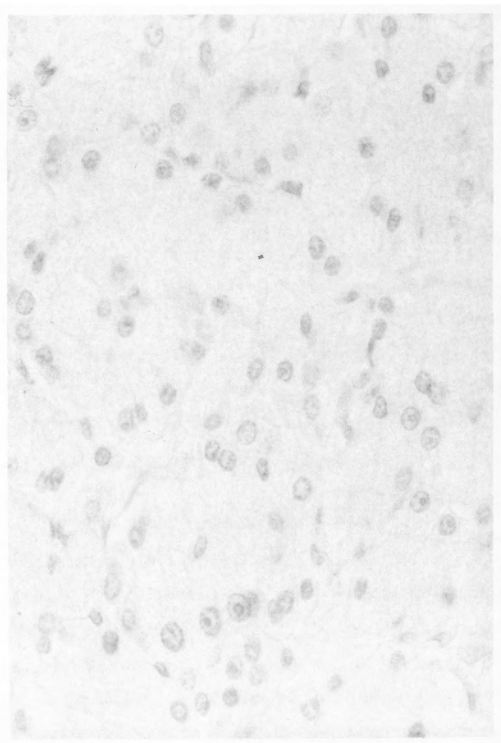


Fig. 3 No immunoreactive product is found in the control sections incubated with normal rabbit serum. (×400)

Results

No significant difference was noted in body weight or non-fasting blood sugar level among the four groups.

After immunohistochemical staining, no immunoreactivity was detected in the control sections incubated with normal rabbit serum (Fig. 3).

In the pancreata treated with saline after sham operation (group A), EGF receptor immunoreactive products were evenly detected in acinar cells, although the intensity of staining was weak (Fig. 4A). However, no immunoreactive products were detectable in islet cells or the cells lining the intercalated ducts. In most acinar cells, the immunoreactive products were homogeneously and faintly distributed in the cytoplasm. Some immunoreactive products appeared to be cytoplasmic granules in some acinar cells, which may be identical to *cytoplasmic granules* reported by DAMJANOV and colleagues[8] (Fig. 4B).

In the pancreata of the rats treated with saline after distal pancreatectomy (group C), the cytoplasmic granular pattern in acinar cells was observed much more intensely and clearly than group A (Fig. 5). Additionally, immunoreactive products against EGF receptor were detected in some cells lining the intercalated ducts and some basal cells of the acinus.

In the pancreata of animals treated with EGF, either after the sham-operation (group B) or after distal pancreatectomy (group D), EGF receptor immunoreactivity was equivalent or even less in-

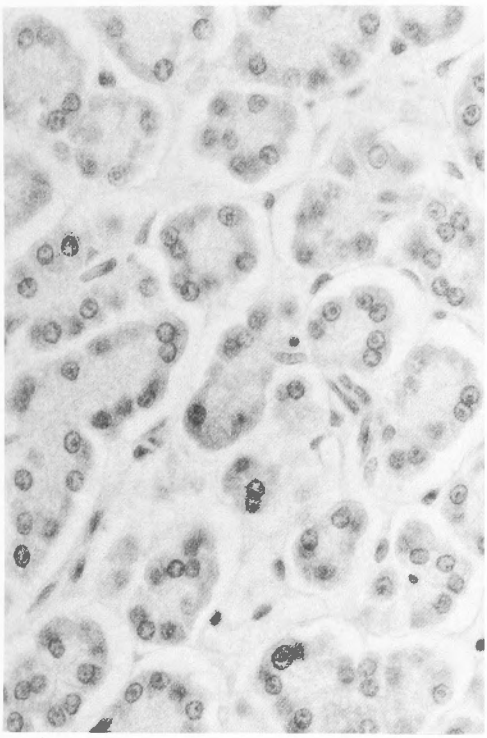


Fig. 4A Immunohistochemistry showing the immunoreactive products (brown) against EGF receptor in the rat pancreas (group A). The tissue was taken on the fifth day after sham-operation. EGF receptor immunoreactive products are only weakly positive in acinar cells. (×400)

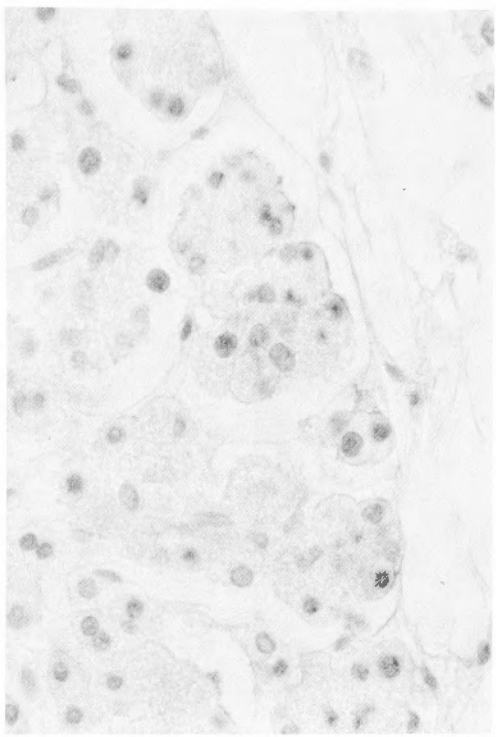


Fig. 4B Another part of the pancreas in group A. Some immunoreactive products are noted as cytoplasmic granules in acinar cells. (×400)

tense than that of group A. The cytoplasmic granular pattern was not found (Fig. 6, Fig. 7).

Discussion

In the present study, it has been shown immunohistochemically that intense EGF receptor immunoreactivity was detected in pancreatic acinar cells after partial pancreatectomy. However, the immunoreactivity was found to be negligible when treated with serial human EGF after partial pancreatectomy.

Brockenbrough and colleagues[4] demonstrated that EGF binding to acini was altered after 90% partial pancreatectomy and that EGF binding was decreased by 35% on the third day after operation with increased mitotic activity. We have also noted an increasing mitotic activity of acinar cells after distal pancreatectomy in our preliminary study[11]. As for the expression of EGF receptor, however, there was some discrepancy between the report by Brockenbrough et al.[4] and ours. The discrepancy between the studies may be explained as follows:

First, in their binding studies[4], they used ^{125}I-labelled EGF which binds unoccupied and accessi-

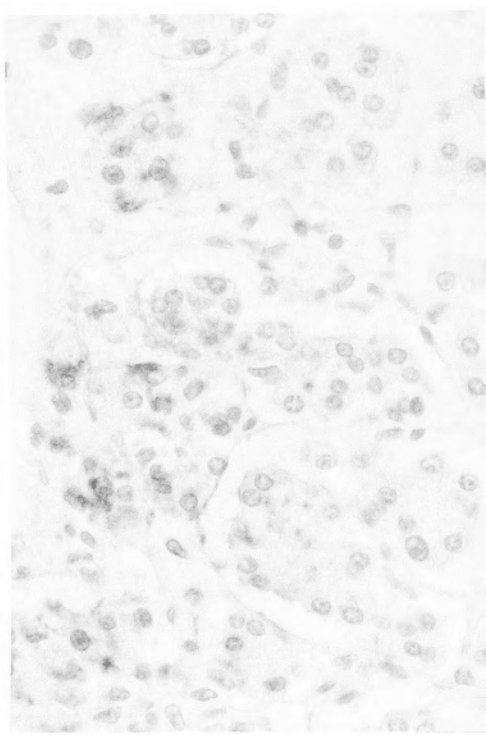


Fig. 5 Immunohistochemistry showing the immunoreactive products against EGF receptor in the rat pancreas (group C). The rat was partially pancreatectomized and injected with saline. Cytoplasmic granular pattern in acinar cells is found more intensely and clearly than in group A. Additionally, immunoreactive products were detected in some cells lining the intercalated ducts and some basal cells of acinus. (×400)

Fig. 6 Immunohistochemistry showing the immunoreactive products (brown) against EGF receptor in the rat pancreas (group B). The rat was sham-operated and treated with EGF. EGF receptor immunoreactive products are equivalent or even less intense than that of group A. (×100)

ble EGF receptor on the cell surface. Because the receptor bound with EGF is assumed to cluster on the plasma membrane and be internalized after autophosphorylation[9], some artificial stimulation or modification for EGF receptor may have occurred in isolated acinar cells. On the contrary, in immunohistochemical studies, the antibody binds EGF receptor without modifying the condition of EGF receptor. In addition, the anti-EGF receptor antibody we used in this study was raised against the autophosphorylation site that was in the intracellular domain of EGF receptor (Fig. 1), and do not recognize the active binding site of EGF receptor. Thus significant difference exists in the site of EGF receptor recognized in their study and ours.

Although immunohistochemical staining is generally less sensitive than direct binding assays, and does not allow quantitation of the antigen, immunohistochemical study may be useful when a specific site of antigens need to be detected.

Second, BROCKENBROUGH and colleagues[4] used younger male Sprague-Dawley rats weighing between 90 g to 110 g and resected a larger pancreatic portion up to 90% in the wet weight. As DAM-

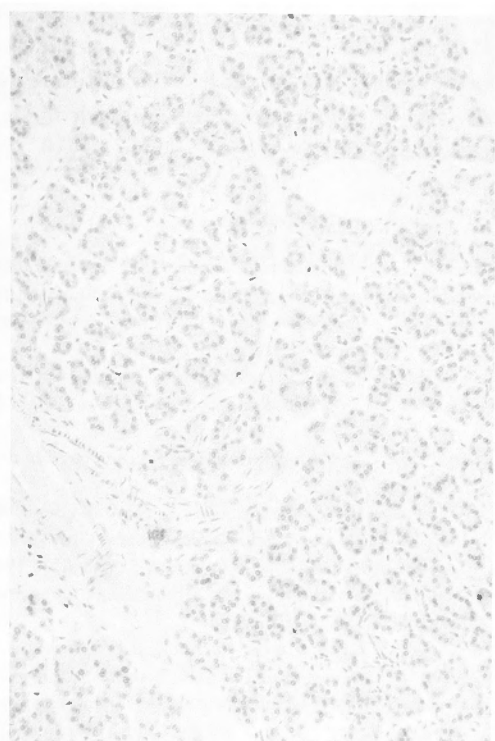


Fig. 7 Immunohistochemistry showing the immunoreactive products against EGF receptor in the rat pancreas (group D). The rat was partially pancreatectomized and injected with EGF. EGF receptor immunoreactive products are equivalent or even less intense than that of group A. (×100)

JANOV and colleagues[8] mentioned, once cells have differentiated into a non-proliferating state, EGF receptor is reduced to undetectable levels or even extinguished. It may be possible that age of animals affects the expression of EGF receptors. EGF receptor may be more readily inducible in younger rats. Although some studies concerning the EGF binding sites in the rat pancreas have been undertaken using binding methods, and have proven the presence of EGF receptors in pancreatic acini[6,16], the age effect on the expression of EGF receptor is unknown. PEARSON and colleagues[19] have observed that the extent of regeneration was positively correlated with the degree of pancreatic resection. It may be also possible to speculate that the degree of resection affects the expression of EGF receptors.

The weak immunoreactivity in the acinar cells of group D may be explained by the down regulation of EGF binding by exogenous EGF[14]. But it must be elucidated whether exogenous EGF really affects the expression of EGF receptor in three cells.

In summary, this study demonstrates that EGF receptors are intensively expressed in the adult rat pancreas after partial pancreatectomy, but disappear when exogenous EGF is consecutively given. Though little information is available about the intracellular mechanism and regulation of pancreatic cells after EGF bindings to the receptor, these results suggest that EGF may exert inhibitory effects on the expression of EGF receptor during regeneration of adult rat pancreas, and that EGF could be involved in the regenerating process as an important factor.

Acknowledgment

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和文抄録

成熟ラットにおける膵部分切除後の上皮細胞増殖因子受容体の発現

——免疫組織学的検討——

京都大学医学部第一外科

東出　俊一，井上　一知，蓑手　秀司，戸部　隆吉

同第一解剖

森　　千里，塩田　浩平

上皮細胞増殖因子 (EGF) の受容体に対する特異的抗体を用いて，成熟ラット膵における膵部分切除後のEGF 受容体の発現状態を免疫組織化学的に検討した．単開腹を施行した対照群においては，膵腺房細胞内にのみ免疫反応産物が認められた．

他方，膵湿重量に対して約50%の膵部分切除術を施行した群においては，切除後5日目における検討にて，膵腺房内に対照群と比較して強い免疫反応産物を認め，さらに一部の腺房周囲の上皮細胞にも反応産物を認めた．しかしながら，膵部分切除術施行後 EGF 筋注投与(100 μg/kg，3回／日×3日間)を加えること，この免疫反応産物は認められなかった．以上より，成熟ラット膵において，膵部分切除後に EGF 受容体の発現が強く惹起され，さらに，外因性 EGF の投与により抑制されることが示唆された．EGF が膵切除後の再生過程に関与している可能性を示唆するものと考えられた．